(245 vs. 104, P = 0.004) and received more treatments (13 vs. 8, P = 0.006), compared with non-classic cGVHD. The steroid dose (mg/kg) prior to ECP was lower in classic cGVHD (0.43 vs. 1.21, P < 0.001). 25 of 38 pts. (66%) with classic cGVHD were on ≤1 mg/kg of prednisone at ECP start, and 14/17 (82%) of pts. with non-classic cGVHD were on > 1 mg/kg of steroids (P = 0.001). For the entire cohort, the steroid dose at month 2 of ECP was significantly less (0.81 vs. 0.38, P = 0.004). In pts. with classic cGVHD, there was a trend in decrease in skin subscale scores after 2 months of ECP. In univariate analysis, OS was superior for classic cGVHD compared with other subtypes (median survival not reached vs. 78 days, P < 0.001; 1-yr OS 65% vs. 10%). OS was better for pts. with steroid dose ≤ 1 mg/kg at start of ECP compared with pts. on higher dose steroid (median survival not reached vs. 69 days, P < 0.001, 1-yr OS 65% vs. 0%). Using Cox regression (adjusted for steroid dose) non-classic cGVHD was an independent prognostic feature for poor survival (HR 4.72, 95% CI 1.84–12.41, P = 0.001). **Conclusion:** Pts. with classic cGVHD had a superior survival after ECP compared to other NIH subtypes. Survival after ECP with other GVHD subtypes is poor and combination of novel steroid sparing agents with ECP need to be explored.

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THYMOGLOBULIN BINDS NATURAL KILLER CELLS AND INDUCES ACTIVATION AND INTERFERON-γ PRODUCTION

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Background: Antithymocyte globulin (Thymoglobulin, rabbit IgG) is widely used in hematopoietic stem cell transplantation (HSCT) to prevent rejection and graft-versus-host disease (GVHD). A beneficial effect of natural killer (NK)-cell alloreactivity on HSCT outcome has been described, but only in patients receiving ATG. We therefore investigated the *in vitro* effects of ATG on purified NK cells. **Methods:** ATG binding to human NK cells and their activation status were assessed by flow cytometry. NK surface targets for ATG were determined by competition inhibition assays using monoclonal antibodies. Chromium 51 (^{51}Cr) release assay, annexin V combined to 7AAD staining and CFSE staining were used to study cytotoxic activity, apoptosis/cell death and proliferation of NK cells, respectively. Interferon (IFN)-γ production was determined by ELISA. **Results:** ATG, ATG-derived Fab'2 fragments as well as rabbit IgG bound NK cells, and competed strongly with anti-CD16. ATG enhanced the expression of activation (CD69, NKG2D) and degranulation (CD107a) markers on NK cells. ATG competed with CD18 binding and decreased NK cytotoxicity, which was restored by IL-15. No effects on apoptosis/cell death and proliferation were observed. Interestingly, ATG, ATG-derived Fab'2 fragments as well as rabbit IgG strongly induced IFN-γ production. **Conclusions:** ATG binds NK cells via CD16 by its variable and constant regions. The decrease in NK cytotoxic activity *in vitro*, which may be explained by CD18 binding, is restored by IL-15, and contrasts sharply with the induction of activation, degranulation and IFN-γ production. These data support the hypothesis that ATG treatment is required to observe the beneficial effect of NK cell alloreactivity in HSCT.

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EXTRACORPOREAL PHOTOCHEMOTHERAPY (ECP) FOR STEROID-REFRACTORY GRAFT-VERSUS-HOST DISEASE (GVHD) IN LOW-WEIGHT PEDIATRIC PATIENTS. CHANGES IN L-SELECTIN EXPRESSION BY T LYMPHOCYTES AND CLINICAL OUTCOME

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Extracorporeal photochemotherapy (ECP) is an emerging treatment modality for steroid-refractory GVHD. The mechanisms by which ECP works are still not fully understood, and modulation of dendritic cell subpopulations, a shift of cytokine profile from Th1 to Th2 and an increase of T-cell regulatory (Treg) cells have been related to the ECP beneficial effect. We analyzed the clinical outcome and the effect of ECP on the T lymphocyte subsets of 11 children with steroid-refractory GVHD. ECP was performed by a continuous-flow cell separator (COBE Spectra). We studied the L-selectin (CD62L) and the CD45RA expression on CD4 and CD8 lymphocytes by flow cytometry. We compared the proportion of each of these 4 subpopulations, as well as the L-selectin positive and L-selectin negative ones, in samples collected from peripheral blood before the first (PRE) and after the last (POST) ECP procedures. Results are shown in Table 1.

Complete response (CR) was achieved in six cases. Skin involvement responded in all cases. ECP was associated with minimal side effects. There was a significant increasing in the CD4/CD8 ratio. Central memory (CM) cells decreased and effector memory (EM) cells increased with ECP in CD4 and CD8 T–cell subsets. The proportion of L-selectin expressing T lymphocytes significantly diminished after ECP, both in CD4 and in CD8 cells. L-selectin is an important T-cell homing receptor for T-cell entry into lymph nodes via high endothelial venules. Expression of CD62L is rapidly lost following T-cell receptor activation, leading to exit from the lymph node into the periphery and sites of inflammation. $CD62L^{neg}$ and $CD62L^{pos}$ also differ in their functional abilities, such as cytokine secretion and cytolytic potential.

Our results suggest that ECP may have an impact in the trafficking patterns of T lymphocytes, redirectioning T cells from lymphoid to extralymphoid organs.

CD4 and CD8 subsets pre and postECP

	PRE	POST	p value
CD4 subsets			
TN (CD62L+CD45RA+)	6.58 ± 2.39	6.18 ± 2.96	0.2
TCM (CD62L+CD45RA-)	58.17 ± 4.49	43.85 ± 4.78	0.02
TEM (CD62L-CD45RA-)	34.64 ± 4.51	46.86 ± 4.89	0.03
TD (CD62L-CD45RA+)	0.6 ± 0.18	3.11 ± 1.46	0.17
CD62Lpos	64.76 ± 4.4	50.03 ± 5.45	0.02
CD62Lneg	35.23 ± 4.4	49.97 ± 5.45	0.02
CD8 subsets			
TN (CD62L+CD45RA+)	16.95 ± 3.94	12.53 ± 4.05	0.23
TCM (CD62L+CD45RA-)	28.8 ± 4.95	12.27 ± 2.86	0.001
TEM (CD62L-CD45RA-)	46.62 ± 5.79	51.4 ± 4.22	0.14
TD (CD62L-CD45RA+)	11.17 ± 3.34	23.52 ± 4.79	0.01
CD62Lpos	45.75 ± 6.1	24.8 ± 5.34	0.01
CD62Lneg	53.8 ± 6.07	74.92 ± 5.31	0.01

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REDUCED RELAPSE RELATED DEATH (RRD) IN ALTERNATIVE DONOR TRANSPLANTS WITH EBV/CMV REACTIVATION

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Viral infections are a serious diagnostic and therapeutic problem in patients undergoing alternative donor transplants. We have analyzed 179 transplants with hematologic malignancies, ghrafted from unrelated (n = 139) or family mismatched donors (n = 40): Patients in this study were alive on day +30 and had been monitored weekly for cytomegalovirus (CMV) reactivation by CMV-antigenemia, and for epstein barr virus (EBV) reactivation by real time PCR. All patients received anti-thymocyte globulin 7.5–11 mg/kg for GvHD prophylaxis together with cyclosporin and methotrextae. The conditioniong regimen was a conventional CY-TBI (n = 102) or a reduced intensity (RIC) thiotepa based regimen (n = 77). The diagnosis was acute leukemia (n = 126) or chronic lymphoid or myeloid disorder (n = 53).

Median age was 36 years (11–64) and transplants were performed between 2000 and 2006. Reactivation of CMV was seen in 78 patients (44%); EBV reactivation in 118 patients (66%). The average time to CMV reactivation was day 49 (95%CI day 29–69) and for EBV it was day +78 (95%CI day 50–105). With an average follow up of 823 days (95%CI 717–929) the overall actuarial 5 year survival